Green Tea Inhibits Vascular Endothelial Growth Factor (VEGF) Induction in Human Breast Cancer Cells

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ABSTRACT Investigators have shown that green tea and its main catechin epigallocatechin-3 gallate (EGCG) may decrease the risk of cancer. Our previous study showed that green tea extract (GTE) as well as its individual catechin components inhibited MDA-MB231 breast cancer cell and human umbilical vein endothelial cell (HUVEC) proliferation. Further, GTE suppressed breast cancer xenograft size and decreased the tumor vessel density in vivo. In the current study, we investigated the effect of GTE on the major angiogenic factor vascular endothelial growth factor (VEGF) in an in vitro experiment. GTE or EGCG (40 mg/L) significantly decreased the levels of the VEGF peptide secreted into conditioned media. This occurred in both HUVEC and human breast cancer cells and the effect was dose dependent. Furthermore, GTE and EGCG decreased the RNA levels of VEGF in MDA-MB231 cells. This inhibition occurred at the transcriptional regulation level and was accompanied by a significant decrease in VEGF promoter activity. We also showed that GTE decreased c-fos and c-jun RNA transcripts, suggesting that activator protein (AP)-1–responsive regions present in the human VEGF promoter may be involved in the inhibitory effect of GTE. Furthermore, GTE suppressed the expression of protein kinase C, another VEGF transcription modulator, in breast cancer cells. Inhibition of VEGF transcription appeared to be one of the molecular mechanisms involved in the antiangiogenic effects of green tea, which may contribute to its potential use for breast cancer treatment and/or prevention. J. Nutr. 132: 2307–2311, 2002.

KEY WORDS: • green tea • vascular endothelial growth factor • epigallocatechin-3 gallate • breast cancer • angiogenesis.

Many epidemiologic studies have shown that green tea may decrease the risk of cancer (1). A distinction should be made between green tea and black tea. Most reports showing positive cancer preventive effects are from studies of Asians who drink predominantly green tea (2), whereas studies of Europeans who drink black tea only infrequently found protective effects (3). The cancer preventive effect of green tea is observed in pancreatic, colon and rectal cancers (4). Some other studies support this phenomenon in colorectal cancer but others do not (5–7). Stomach cancer has been studied with mixed results (8), but it seems that high consumption of tea at moderate temperature is beneficial (9–12). Green tea has been observed to decrease the risk of esophageal cancer (13). The effect on lung cancer is not definitive (14,15). Finally, there are reports linking the consumption of green tea with an improved prognosis in breast cancer (16–18).

The mechanisms of the cancer preventive effect of green tea are being explored. Recently, we examined the effect of green tea on the growth of breast cancer and endothelial cells in both in vitro assays and animal models. We demonstrated that both green tea extract (GTE)4 as well as its individual catechin components were effective in inhibiting breast cancer and endothelial cell proliferation. In mouse experiments, GTE suppressed xenograft size and decreased the tumor vessel density (19). In the present study, we investigated the effects of GTE and epigallocatechin-3-gallate (EGCG) on angiogenic factor vascular endothelial growth factor (VEGF) expression with in vitro studies.

MATERIALS AND METHODS

Reagents. GTE was obtained from Pharmanex batch #990222 (Brisbane, CA). GTE was characterized by HPLC, and its catechin components were described recently (19). EGCG and phorbol myristate acetate (PMA) were purchased from Sigma Chemical (St. Louis, MO).

Cell culture. Human umbilical vein endothelial cell (HUVEC) was purchased from Cascade Biologics (Portland, OR). The cells were plated on tissue culture flasks coated with 1.5% gelatin (Difco, 0022-3166/02 $3.00 © 2002 American Society for Nutritional Sciences.
formed. Brie-tractor from cells using the Trizol Reagent (GIBCO BRL Life Tech-
overs various time durations of treatment were collected, centrifuged
lected as negative controls. After cells were incubated in the trans-
structs were cotransfected into cells with the FuGENETM6 Trans-
VEGF secretion and peptide levels increased as the culture
effects on VEGF peptide secretion, but there was no dose by
tively determined as described by the manufacturer
RA extraction and Northern analysis. Total RNA was ex-
tulated to Micro Cart (Amersham). The probed nylon membranes were then washed and
are different from each other. Western analysis. To evaluate protein kinase C (PKC) protein
s level and total protein concent-
styles were prepared for both VEGF level and total protein concent-
determined at 450 nm using a microtiter plate spectrophotom-
EGCG on VEGF secretion into condi-
VEGF promoter activity. We then examined the effect of GTE and
influence the activity of VEGF promoter activity secondary to
the effect of GTE on c-fos expression, MDA-
VEGF promoter activity was determined with a sin-
they were cultured for 48 h in the
had signifi-
can be measured by a colorimetric assay (Bio-Rad, Hercules, CA). VEGF
obtained minimum essential medium (DMEM; Life Technologies, Grand
serum (FCS), penicillin, streptomycin, and amphotericin-B (Cascade Bio-
GTE- and EGCG-treated MDA-MB231 hu-
strongly linked to the
and 100 mg/L streptomycin. The conditioned media
the Bradford method using a colorimetric assay (Bio-Rad, Hercules, CA). VEGF
transfection assay is a useful tool for examining gene
A

RESULTS

Effect of GTE on VEGF secretion into conditioned media of human breast cancer cells and HUVEC.
The control and GTE- and EGCC-treated MDA-MB231 hu-

Effect of GTE or EGCG on VEGF transcripts in human breast cancer cells. We then examined the effect of GTE and

Effect of GTE on basal and PMA-stimulated c-fos and c-jun transcripts in human breast cancer cells. To evaluate the effect of GTE treatment on c-fos expression, MDA-

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absence or presence of 40 mg/L GTE. At the end of this period, cells were or were not stimulated by 50 nmol/L PMA for 30 min. GTE-treated MDA-MB231 cells had lower basal and PMA-stimulated c-fos RNA levels than control cells (Fig. 5). The same phenomena were observed for c-jun expression in these cells (Fig. 6).

Effect of GTE on PKC protein levels in human breast cancer cells. MDA-MB231 cells were cultured for 48 h with or without GTE or EGCG. As shown in Figure 7, both GTE and EGCG decreased PKC protein levels.

DISCUSSION

This study provides further evidence that green tea extract inhibits breast cancer angiogenesis. In our previous study, we demonstrated that both green tea extract as well as its individual catechin components were effective in inhibiting breast cancer and endothelial cell proliferation. In mouse experiments, green tea extract suppressed xenograft size and decreased the tumor vessel density (19). Yang et al. (23) recently reviewed the anticancer properties of green tea constituents.

Because all solid tumors are angiogenesis dependent, the suppression of endothelial cells would contribute to the overall tumor inhibition. Swiercz et al. (24) observed that EGCG reduced angiogenesis in the chicken embryo chorioallantoic membrane model. Cao and Cao (25) showed that EGCG inhibited bovine capillary endothelial cells in culture, with a 50% reduction at an EGCG concentration of 50 mg/L. In other published studies, green tea increased the frequency of apoptotic endothelial cells in a mouse model of human non-Hodgkin’s lymphoma (26).

Angiogenesis is a complex process with many steps involving soluble factors, adhesion molecules, proteases and cytokines. Recent reports of the effect of known angiogenic growth factors on the endothelium have advanced our understanding of the mechanisms of angiogenesis at a molecular level. The best-studied angiogenic growth factor is VEGF (27). VEGF binds to the receptor VEGF-R2 (Flk1) and sends a classical
proliferative signal to the endothelial cell. Subsequently, VEGF binding to another receptor, VEGF-R1 (Flt1), elicits endothelial cell-cell interactions and capillary formation.

In our present study, we demonstrated that 40 mg/L GTE or EGCG greatly inhibits VEGF protein secretion in conditioned media. This inhibition occurred at the level of transcriptional regulation, which was manifested by a decrease in transcript levels and a decrease in VEGF promoter activity. This phenomenon was recently observed in colon cancer cells (22). The VEGF promoter region contains several potential binding sites for the transcription factor activator protein-1 (AP-1) (28), and c-fos and c-jun are components of the AP-1 transcription factor complex (29). Our data showed an inhibition of c-fos and c-jun expression in both unstimulated and PMA-stimulated MDA-MB231 cells by GTE. We further explored PKC, another modulator of VEGF expression (30). PKC expression in breast cancer cells was inhibited by both GTE and EGCG. These results are consistent with reports in other cell types of PKC and AP-1 inhibition exerted by various tea components (31–33).

The inhibition of breast cancer angiogenesis by green tea likely involves multiple pathways other than VEGF transcrip-
tion. It has been reported in other cell types that green tea inhibits other angiogenic molecules, i.e., urokinase (34), matrix metalloproteinases (MMP-2 and MMP-9) (35), and platelet-derived growth factor (36). Tumor necrosis factor-α gene expression has also been shown to be inhibited by EGCG (17). A recent abstract suggested that another mechanism involves the suppression of interleukin-8 production by endothelial cells (37).

The observation that green tea is antiangiogenic is very important clinically. Currently, there is much effort to develop antiangiogenic drugs to treat cancer. Many of these agents have completed Phase I clinical trials, and are currently in Phases II and III (38). A major shortcoming of the vast majority of the antiangiogenic drugs is that they require intravenous or subcutaneous administration. This is particularly problematic because antiangiogenic drugs have to be given on a long-term basis to control cancer growth. Furthermore, many of these compounds are complex peptides that are diffi-
cult and expensive to produce in the quantities and purities required for human use. Thus, an antiangiogenic agent that can be administered orally and inexpensively, such as green tea, would be clinically very useful. To date, a recent report of a Phase I clinical trial of green tea has been encouraging (39).

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LITERATURE CITED